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(54) Process for preparing 2,5-Diketogluconic Acid

(57) A process for producing 2,5-diketogluconic acid which comprises aerobically propagating *Acetobacter cerinus* in a glucose medium and then recovering the resulting 2,5-diketogluconic acid or processing the fermentation broth by selective reduction to yield 2-ketogulonic and 2-ketogluconic acids.

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SPECIFICATION

Process for Preparing 2,5-Diketogluconic acid

This invention relates to the preparation of 2,5-diketogluconic acid by fermentation.

2,5-Diketogluconic acid is a useful intermediate in the synthesis of vitamin C. Heretofore, 2,5-diketogluconic acid has been produced by several different varieties of bacteria such as *Acetobacter melanogenum, Acetobacter aurantium, Gluconoacetobacter rubiginosus, Gluconoacetobacter liquifaciens* and *Pseudomonas sesami*. The use of these micro-organisms, however, is not completely desirable from an industrial point of view because of the production of large amounts of brown or yellowish-brown pigments as by-products of cultivation, thereby decreasing the purity of the co-produced 2, 5-diketogluconic acid.

U.S. Patent 3,790,444 claims the production of 2,5-diketogluconic acid, without accompanying brown pigment, by a new species designated *Acetobacter fragum*.

This invention is concerned with an economical process for preparing 2,5-diketogluconic acid by the use of readily available, publicly held strains of *Acetobacter cerinus*. Two of these strains, IFO 3263 and 3266, produce 2,5-diketogluconic acid in yields of >95% (based on glucose).

2,5-Diketogluconic acid is useful as an intermediate for the preparation of ascorbic acid. An aqueous solution of 2,5-diketogluconic acid may be selectively reduced to provide a mixture of 2-ketogulonate and 2-ketogluconate which can be converted to ascorbic and erythorbic acids.

2,5-Diketogluconic acid is readily prepared by bacterial action on glucose employing, according to the process of the present invention, readily available strains of *Acetobacter cerinus*. All of the listed publicly held strains of *Acetobacter cerinus* have been tested and shown in this investigation to produce keto-acids at a yield of 50–95% (based on glucose). When *Acetobacter cerinus* IFO 3263 or 3266 is employed, the keto-acid produced is entirely desired 2,5-diketogluconic acid in yields of >95% (based on glucose).

The available, publicly held strains of *Acetobacter cerinus* are as follows:

IFO 3262 (ATCC 12303)
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These strains of *Acetobacter cerinus* are cultured in a medium of which the main carbon source is glucose. These micro organisms do not require expensive organic nitrogen sources such as peptone or meat extract. When urea and inorganic nitrogen sources such as ammonium sulfate, ammonium nitrate or ammonium phosphate are employed, nicotinic acid is added as an essential growth factor.

The glucose concentration in the medium varies between 2.5 and 20% preferably between 10 and 12%, in order to obtain 2,5-diketogluconic acid most economically. The fermentation temperature is between 20 and 35°C, preferably between 25 and 30°C, most preferably around 28°C. The initial pH of the culturre medium may range from 3.5 to 7.5, preferably at 5 to 6.

During the course of the fermentation, the pH is maintained at about 5.5 by the addition of sodium hydroxide solution. Calcium carbonate may be used for pH control and is added in medium make-up after autoclaving at an amount of 30 grams per 110 grams of glucose.

After inoculation, the fermentation medium is agitated with a mechanical stirrer at about 1700 r.p.m., and aerated at the rate of 0.5 to 1 volume of air per volume of broth per minutes.

Employing *Acetobacter cerinus* IFO 3263 or 3266, the fermentation is conducted until a yield of 2,5-diketogluconic acid of at least 90% (based on glucose) is obtained (36 – 40 hours).

It was determined by paper chromatography that the conversion of glucose to 2,5-diketogluconic is via the following pathways:

Glucose→2-ketogluconic acid→2,5-diketogluconic acid

Glucose→5-ketogluconic acid→2,5-diketogluconic acid

Whatman No. 1 and No. 4 paper are used employing a solvent system of methylethyl ketone acetone formic acid: water (80:6:2:12). The acid spots are located by spraying with a 0.2% o-phenylenediamine ethanolic solution containing 1% nitric acid and heating to about 70°C, (5-ketogluconic acid – blue; 2-keto-gluconic acid – yellow; 2,5-diketogluconic acid – green). High pressure liquid chromatography may also be used for identification.

2,5-Diketogluconic acid may be separated and recovered from the final fermentation broth by any conventional procedure known to those skilled in the art.

The filtered fermentation broth may be processed as is by treatment with a borohydride and the resultant mixture of 2-ketogluconic acid and 2-ketogulconic acid hydrolyzed to yield ascorbic and erythorbic acids as described in co-pending British Patent Application No. 51415/77 21.3.79.

Example 1

The following aqueous inoculum medium was prepared:

Ingredient	Grams/litre
Glucose	25
Corn steep liquor	5
KH_2PO_4	0.5
K_2HPO_4	0.5
$MgSO_4 \cdot 7H_2O$	0.2
$CaCO_3$	6.3

pH 6.2

A shake flask containing one litre of medium was autoclaved for 30 minutes at 121°C. The pH of the cooled medium was 5.0. Cells of *Acetobacter cerinus* IFO 3263 from a nutrient agar slant (5 ml of a 20 ml sterile aqueous suspension) were added to the flask which was then shaken on a rotary shaker at about 28°C for about 24 hours.

An aliquot of the culture growth sufficient to provide a 5% v/v inoculum was added to a 4-litre stirred fermentor containing 2 litres of the following production medium:

Ingredient	Grams/litre
Glucose	110
Corn steep liquor	0.5
$(NH_4)_2HPO_4$	0.58
KH_2PO_4	1.5
$MgSO_4 \cdot 7H_2O$	0.5
Urea	0.5
$CuSO_4 \cdot 5H_2O$	1 mg
Nicotinic acid	300 γ

pH 6.0

The fermentation was conducted at a temperature of about 28°C with stirring at 1700 r.p.m. and aeration at the rate of 0.75 volume per volume of broth per minutes. After a fermentation period of about 20 hours, sterile aqueous glucose was added (55 grams/lite). The pH was maintained at 5.5 by the addition of sodium hydroxide solution. The fermentation was continued until a yield of 2,5-diketogluconic acid of 95% (based on glucose) was obtained.

Example 2

The method of Example 1 may be repeated with comparable results employing *Acetobacter cerinus* IFO 3266.

CLAIMS

1. A process for producing 2,5-diketogluconic acid which comprises aerobically propogating *Acetobacter cerinus* in a glucose medium and then recovering the resulting 2,5-diketogluconic acid or processing the fermentation broth by selective reduction to yield 2-ketogulonic and 2-ketogluconic acids.
2. A process as claimed in claim 1, wherein the glucose concentration in the medium is form 2.5 to 20%, the fermentation temperature is from 20 to 35°C, the initial pH is from 3.5 and 7.5, and the pH during the course of the fermentation is maintained at about 5.5.
3. A process as claimed in claim 1 or 2 wherein said *Acetobacter cerinus* is strain IFO 3263.
4. A process as claimed in claim 1 or 2 wherein said *Acetobacter cerinus* is strain IFO 3266.
5. A process as claimed in claim 1 substantially as described in Example 1 or 2.
6. 2,5-Diketogluconic acid, 2-ketogulonic or 2-ketgluconic acid, which has been prepared by a process as claimed in any one of claims 1 to 5.

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